

I/WE CLAIM:

1. A method for determining depth of a volume comprising a fluorophore in a turbid medium using time domain(TD) optical fluorescence, said method
5 comprising:
 - i) obtaining at least one temporal point spread function (TPSF) by injecting light at an injection point at an excitation wavelength of said fluorophore and detecting light at a
10 detection point at an emission wavelength of said fluorophore;
 - ii) determining a time t_{\max} substantially corresponding to the maximum of said TPSF;
 - iii) correlating said t_{\max} with said depth to
15 determine the depth, and wherein said depth is insensitive to fluorophore concentration.
2. The method as claimed in claim 1 wherein said step of correlating comprises:
 - a) establishing a calibration curve of t_{\max} as a
20 function of depth for a plurality of depths;
 - b) using said calibration curve and said t_{\max} determined in step ii) to obtain said depth of said volume.
3. The method as claimed in claim 1 wherein said
25 injection and detection points are in a back-reflection geometry.

4. The method as claimed in claim 3 wherein said injection and detection points are substantially equidistant from said volume.
5. The method as claimed in claim 4 wherein said step of correlating comprises:
 - a) providing scatter coefficient, speed of light in said medium and a lifetime of said fluorophore in said medium;
 - b) calculating said depth using said t_{\max} and said scatter coefficient, said speed of light and said lifetime.
6. The method as claimed in claim 4 or 5 further comprising a step of estimating a position of said fluorophore in a plane substantially perpendicular to said depth prior to said step of obtaining said TPSF.
7. The method as claimed in claim 6 wherein said step of estimating is performed by obtaining a topographic image of a region of interest containing said fluorophore.
8. The method as claimed in any one of claims 1-7 wherein said fluorophore is an intrinsic or extrinsic fluorophore.
9. The method as claimed in any one of claim 5-8 wherein said scatter coefficient and lifetime are

obtained using time domain optical measurements of said medium.

10. The method as claimed in any one of claim 5-8 wherein said scatter coefficient, lifetime and speed of light are obtained by matching said medium with a similar medium in a database for which optical properties are known.
11. The method as claimed in any one of claim 5-10, wherein said scatter coefficient and said speed of light are substantially the same at both the excitation or emission wavelength of the fluorophore and are determined at either said emission or said excitation wavelength.
12. The method as claimed in any one of claim 5-10, wherein said scatter coefficient and said speed of light are determined at the fluorophore excitation and emission wavelength.
13. A method for estimating concentration of a fluorophore in a volume in a turbid medium using optical fluorescence, said method comprising:
- i) obtaining depth of said volume using the method of any one of claim 1-12;
 - iii) obtaining a fluorescence emission intensity of said fluorophore; and
 - iv) deriving said concentration from a fluorescence emission equation.

14. The method as claimed in claim 13 wherein said emission intensity is obtained in a back-reflection configuration.
15. The method as claimed in claim 13 wherein said
5 fluorescence emission intensity is obtained in a trans-illumination configuration.
16. The method as claimed in claim 14 or 15 wherein said emission intensity is obtained in a modality selected from time domain, frequency domain and
10 continuous wave.
17. The method as claimed in any one of claim 13-16 wherein said equation provides concentration of a fluorophore as a function of depth, scatter coefficient and absorption coefficient.
- 15 18. The method as claimed in claim 17 wherein said coefficients are obtained using time domain optical measurements of said medium.
19. The method as claimed in claim 17 wherein said coefficients are obtained by matching said medium
20 with a similar medium from a database for which the optical properties are known.
20. The method as claimed in any one of claim 17-19, wherein said coefficients are substantially the same at both the excitation or emission wavelength
25 of the fluorophore and is determined at either said emission or said excitation wavelength.

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21. The method as claimed in any one of claim 17-19, wherein said at least one optical property is determined at the fluorophore excitation and emission wavelength.
- 5 22. The method as claimed in any one of claim 13-21 wherein said concentration is a relative concentration.
23. The method as claimed in any one of claim 13-21 wherein said concentration is an absolute
10 concentration derived by calibrating said intensity.
24. A method for generating a tomographic image of a fluorophore distribution in a turbid medium said method comprising:
- 15 i) obtaining a topographic image of said fluorophore distribution;
- ii) determining depth of a plurality of volumes of interest comprising said fluorophore using the method as claimed in any one of
20 claim 1-12;
- iii) combining said depth information and said topographic image to generate a tomographic image of said distribution.
25. The method as claimed in claim 24 wherein said
25 tomographic image is further processed with the method as claimed in any one of claim 13-23 to

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generate a tomographic fluorophore concentration image.

26. A method for determining a relative or absolute concentration of a fluorophore in a turbid medium
5 said method comprising:

- i) establishing a calibration curve relating an emission intensity measurement in said turbid medium and concentration and depth of said fluorophore;
- 10 ii) determining a depth of said fluorophore using the method as claimed in any one of claim 1-12; and
- iii) determining said concentration using said calibration curve.

15 27. The method as claimed in any one of claims 1-26 wherein time domain information is obtained by acquiring Frequency Domain (FD) data and applying a Fourier Transform to said data.

20 28. An apparatus for determining depth and concentration of a fluorophore in a turbid medium comprised within an object, said apparatus comprising:

a light source, optically coupled to a source channel and said object, to inject light in
25 said object at a desired point and excitation wavelength;

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- 5 a first detector channel, optically coupled to a
photon detector and said object, in a back-
reflection geometry relative to said source
channel, to acquire at least one temporal point
spread function from a desired point of said
object to determine depth of said fluorophore;
- 10 a second detector channel in a trans-illumination
geometry relative to said source channel, to
measure an emission intensity of said
fluorophore;
- a means for spatially positioning said object
relative to said channels;
- a depth calculator; and
- a concentration calculator.
- 15 29. The apparatus as claimed in claim 28 wherein said
source and detector channels are in a configuration
selected from contact and free-space optic.
- 20 30. The apparatus as claimed in claim 28 or 29 wherein
said channels comprise optical coupling means
selected from, mirrors, optic fibers.
31. The apparatus as claimed in any one of claim 28-30
wherein said channels are mounted in a fixed
position relative to one another.
- 25 32. The apparatus as claimed in claim 31 wherein said
channels are mounted on a gantry that is moveable
relative to said object.

33. The apparatus as claimed in any one of claim 28-30 wherein said channels are independently moveable.
34. The apparatus as claimed in any one of claim 30-33 wherein said object is placed on a platform
5 transparent to an emission wavelength.
35. The apparatus as claimed in claim 35 wherein said platform is moveable relative to said channels.
36. The apparatus as claimed in any one of claim 28-35 wherein said light source is a multiwavelength
10 light source.
37. The apparatus as claimed in any one of claim 28-36 wherein detection is effected using a plurality of source/detector configurations.
38. The apparatus as claimed in any one of claim 28-37
15 comprising a plurality of detector channels.
39. The apparatus as claimed in any one of claim 28-38 comprising a plurality of source channels.
40. The apparatus as claimed in any one of claim 28-39 further comprising wavelength selection means
20 between said source and said object for selecting one or more excitation wavelength.
41. The apparatus as claimed in any one of claim 28-40 further comprising wavelength selection means

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between said object and said detector for selecting one or more emission wavelengths.

42. The apparatus as claimed in any one of claim 28-41 wherein said detector in a trans-illumination geometry operates in time domain, frequency domain
5 or continuous wave mode.